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1 **Long-term health effects after resolution of acute *Cryptosporidium parvum* infection: a 1-year**
2 **follow-up of outbreak associated cases**

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5 Authors: Rhianwen E Stiff^{a,b}, Angharad P Davies^{a,c}, Brendan W Mason^{d,a}, Hayley A Hutchings^a, Rachel
6 M Chalmers^{c,a}

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8 a. Swansea University Medical School, Singleton Park, Swansea, Wales, SA2 8PP, UK
9 b. Health Protection, Public Health Wales NHS Trust, Temple of Peace, Cathays Park,
10 Cardiff, Wales, CF10 3NW, UK
11 c. Cryptosporidium Reference Unit, Public Health Wales Microbiology, Singleton Hospital,
12 Swansea, Wales, SA2 8QA, UK
13 d. Communicable Disease Surveillance Centre, Public Health Wales NHS Trust, Temple of
14 Peace, Cathays Park, Cardiff, Wales, CF10 3NW, UK
15

16
17 **Corresponding author:**

18 Prof Rachel Chalmers

19
20 Public Health Wales Cryptosporidium Reference Unit/Swansea University Medical School
21 Swansea, Wales
22 SA2 8PP
23 Tel: 01792 285341
24 Fax: 01792 202320
25 Email: rachel.chalmers@wales.nhs.uk
26

27 **Keywords:**

28 *Cryptosporidium*, cryptosporidiosis, long-term health effects, post-acute health effects, sequelae
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32 **Abstract:**

33 We describe a longitudinal study carried out in an adult outbreak-associated cohort to investigate
34 health effects, including post-infectious irritable bowel syndrome, occurring after resolution of acute
35 *Cryptosporidium parvum* infection. New symptoms self-reported up to 12 months included: weight
36 loss (31%), abdominal pain (38%), diarrhoea (33%), eye pain (9%), joint pain (33%), fatigue (22%) and
37 symptoms consistent with irritable bowel syndrome (IBS) (28%). Two people were medically
38 diagnosed with IBS. This study describes for the first time sequelae reported by patients up to twelve
39 months after infection with *C. parvum*, which appear to be similar to those described with *C.*
40 *hominis*.

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51 *Cryptosporidium* is a protozoan parasite which causes symptoms of gastroenteritis including
52 diarrhoea, vomiting and abdominal pain. It is the commonest cause of protozoal diarrhoea in the UK
53 with nearly 6000 laboratory notifications in 2012 (Public Health England, 2014), and it particularly
54 affects children aged between 2 and 5 years. Its global significance has become better recognised
55 since publication of the Global Enteric Multi-centre Study (Kotloff et al., 2013), which found it to be
56 the second commonest pathogen causing moderate to severe diarrhoea in children aged under 1
57 year in low/middle income countries. In immune-competent individuals cryptosporidiosis is often
58 perceived as an unpleasant but relatively mild self-limiting illness. However there is growing
59 evidence to suggest that, like certain bacterial causes of gastroenteritis, it may have longer-term
60 health effects which manifest after resolution of the acute infection. The main two species affecting
61 humans are *C. hominis* (predominantly anthroponotic) and *C. parvum* (both zoonotic and
62 anthroponotic), and as might be expected the epidemiology differs between them. In addition,
63 previous work has suggested that differences may exist in their post-infectious sequelae (Hunter et
64 al., 2004; Bushen et al 2007). However there have been very few studies on this subject.

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66 In the UK, a case-control study of patients who had cryptosporidiosis found that infection with the
67 species *C. hominis* (but not *C. parvum*) was associated with joint pain, eye pains, headaches and
68 fatigue in the two months following infection (Hunter et al., 2004). A seronegative reactive arthritis
69 has been reported in adults (Hay et al., 1987; Ozgul et al., 1999) and children (Shepherd et al., 1989;
70 Cron et al., 1995) including one report of Reiter's syndrome (arthritis, conjunctivitis and urethritis)
71 (Cron et al., 1995). It has also been suggested that *Cryptosporidium* infection may cause Crohn's
72 disease and ulcerative colitis to relapse (Manthey et al., 1997; Colussi et al., 2010; Vadlamudi et al.,
73 2013). A study in Sweden (Rehn et al., 2015) followed up 459 cases who suffered *C. hominis* in two
74 waterborne outbreaks and controls, finding that outbreak cases were more likely to report
75 diarrhoea, abdominal pain and joint pain several months after infection than controls were. In terms
76 of sequelae in developing countries, cryptosporidiosis is now recognized as being associated with

77 stunting of growth, and with persistent diarrhoea. In studies in Brazil (among others), early
78 childhood diarrhoea with *Cryptosporidium* was associated with impaired physical fitness and
79 cognitive function 4-7 years later (Guerrant et al., 1999), and with increased diarrhoea morbidity in
80 subsequent years (Agnew et al., 1998). It is therefore now believed that the effects of
81 cryptosporidiosis go beyond the initial acute diarrhoeal episode, possibly due to an effect on the
82 gastrointestinal epithelium (for example, villous blunting with chronic inflammation, and an
83 association with the poorly understood entity 'environmental enteropathy') (see Bartelt et al.,
84 2013).

85

86 Several agents of infectious gastroenteritis lead to an increased risk of developing irritable bowel
87 syndrome (IBS). IBS is a common condition occurring in about 9-12% of the population in the UK
88 (Jones et al., 2000). A validated diagnostic criteria assessment tool exists for IBS
89 (<http://romecriteria.org/>). Post-infectious IBS (PI-IBS) is well-documented, occurring in 25-38% of
90 patients with enteritis (Jones et al., 2000), for instance after *Campylobacter*, *Salmonella* or *Shigella*
91 infection. People who have had a laboratory confirmed diagnosis of bacterial gastroenteritis are
92 nearly 12 times more likely to develop new-onset IBS in comparison with people who have not had a
93 laboratory diagnosis of bacterial gastroenteritis (Rodriguez et al., 1999). PI-IBS appears to have a
94 rather better prognosis than non-PI-IBS (Jones et al., 2000). For culture-confirmed bacterial
95 gastroenteritis, a RR of 11.9 of new-onset IBS has been reported (Hanevik et al., 2009). This risk is
96 not confined to bacterial infection but has also been documented with *Giardia lamblia* (Hanevik et
97 al., 2009), another protozoan parasite causing gastroenteritis. Infection of a rat model with *C.*
98 *parvum* triggers long-term jejunal hypersensitivity and mast cell accumulation, pathological changes
99 akin to those found in human patients with IBS (Marion et al., 2006; Khaldi et al., 2009).

100

101 During spring 2012, an outbreak of cryptosporidiosis caused by *C. parvum* occurred in the UK (mainly
102 northern England) associated with consumption of pre-cut bagged salad leaves (McKerr et al., 2012).

103 Just over 300 cases were identified and due to the nature of the products involved, most cases were
104 adults. This was a rare opportunity to add to Hunter's evidence (Hunter et al., 2004) relating to the
105 sequelae of infection with *C. parvum* in adults. We undertook a longitudinal study among this adult
106 outbreak-associated cohort to investigate health effects, including PI-IBS, occurring after resolution
107 of acute *C. parvum* infection.

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110 Cases with illness onset between 14 May and 3 June 2012, aged ≥ 16 years, resident in northern
111 England and confirmed as having *C. parvum* infection by the Cryptosporidium Reference Unit were
112 invited to participate in the study. Consenting participants completed a self-administered web-based
113 questionnaire at 6 and 12 months after laboratory diagnosis of cryptosporidiosis (known as 6MQ and
114 12MQ). We sought information about participants' acute illness, as well as their symptoms and
115 medical diagnoses prior to onset of acute cryptosporidiosis and during the following year. A review
116 of previously reported health effects (Hunter et al., 2004; Hay et al., 1987; Ozgul et al., 1999;
117 Shepherd et al., 1989; Cron et al., 1995; Manthey et al., 1997; Rehn et al., 2015) informed our
118 inclusion of questions on specific symptoms and diagnoses, as well as some with no known
119 association, such as diabetes and chest pain, included for comparison. To investigate reported
120 symptoms consistent with a diagnosis of IBS the Rome III diagnostic criteria assessment tool
121 (<http://romecriteria.org/>) was used. Participants were asked about the following conditions
122 diagnosed by a medical practitioner: IBS, ulcerative colitis, Crohn's disease, depression, anxiety,
123 arthritis, diabetes or immunosuppression, either preceding their infection with *Cryptosporidium* or
124 developing in the 12 months afterwards. In addition, data were also collected on self-reported
125 symptoms, either consistent with IBS as indicated by Rome III criteria, weight loss, loss of appetite,
126 nausea, recurrent vomiting, abdominal pain, diarrhoea, blood in stool, blurred vision, eye pain,
127 recurrent headache, dizzy spells, fatigue, joint pain, back pain, fever or chest pain. Persons reporting

128 pre-existing diagnosis or symptoms were excluded when enumerating new diagnosis or symptoms at
129 6MQ and 12MQ.

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131 The results are shown in Table 1. One hundred and ninety seven potential participants were invited,
132 of whom 54 (27%; 14 males and 40 females) took part. Response rates at 6MQ were significantly
133 better among women than men (32.8 % vs 18.7%; difference 14.1 %, 95% CI 1.3% to 25.5%). There
134 was no statistically significant difference in the mean age of those who did (41.8 years) and did not
135 (41.5 years) participate ($p=0.18$). The 12MQ was completed by 39 of the original 54 participants
136 (retention rate 72%). Not all participants responded to each question, resulting in reduced
137 denominators for some responses (Table 1). The mean duration of symptoms of acute
138 cryptosporidiosis was 23 days (range 7-84 days). Six of 54 people spent a total of 95 days in hospital
139 (mean hospital stay 15.8 days $SD\pm 13.3$; range 2-34 days). Self-reported severity was described by
140 32/53 (60%) as severe, 20/53 (38%) as moderate and 1/53 as mild. Among the cohort's 41
141 employees, 434 lost working days were reported. Pre-existing medically diagnosed IBS was reported
142 by 11 of 54 (20%) participants, but this did not appear to correlate with the severity of acute
143 cryptosporidiosis. Those with pre-existing IBS reported a mean duration of illness of 24.8 days
144 ($SD\pm 21.5$; range 14-84 days); 2 of 11 were hospitalized for 2 and 5 days respectively; and 55% self-
145 reported their acute episode as severe. Seven (66%) reported their IBS symptoms were unchanged
146 at 6MQ; 1 reported IBS symptoms had improved; 1 reported IBS symptoms had worsened and 2
147 reported 'not applicable'. At 12MQ, 4 of 9 (44%) responders with pre-existing IBS reported a
148 worsening in their IBS symptoms. At 6MQ, 4 of 43 (9%) people without pre-existing IBS reported
149 symptoms which fulfilled all Rome III criteria for diagnosing IBS, including one person who received a
150 new IBS diagnosis from a doctor. Combining reports from 6MQ and 12MQ, a total of six (14%)
151 responders had symptoms meeting all Rome III criteria, and a total of two people received a new
152 medical diagnosis of IBS. Additionally, new onset gastrointestinal and non-gastrointestinal
153 symptoms were reported at 6MQ and/or 12MQ by a high proportion of participants including

154 weight loss (31%), abdominal pain (38%), diarrhoea (33%), eye pain (9%), joint pain (33%), fatigue
155 (22%) and dizzy spells (10%) (Table 1).

156

157 This outbreak provided an unusual and fortuitous opportunity to recruit a cohort of adults with *C.*
158 *parvum*, since a large proportion of *Cryptosporidium* cases usually occur in young children.

159 Recruitment delay may have biased towards over-representation of those most adversely affected
160 by, or those who attributed post-acute symptoms to, acute cryptosporidiosis. Hunter et al. (2004)

161 reported new or worsened symptoms following cryptosporidiosis in 40.9% of case patients, including
162 statistically significantly more cases than controls with weight loss (29.5%), appetite loss (23%),

163 abdominal pain (26.2%) and diarrhoea (29.5%) at two months after acute infection. A study of a

164 waterborne cryptosporidiosis outbreak in Milwaukee (Manthey et al., 1997) found that 39% of cases
165 experienced a relapse of diarrhoeal symptoms after initial resolution, but this study did not follow

166 patients up for longer than a maximum of three months, and in most cases for less than that.

167 Evidence pertaining to periods longer than this is very scant. One study of cryptosporidiosis in
168 Sweden (without controls) found persistent gastrointestinal symptoms in up to 8 of 53 (15%)

169 patients 25-36 months post-infection: of interest, in this group, no difference was noted between
170 cases who had been infected with *C. hominis* and those with *C. parvum* (Insulander et al., 2013).

171 Another larger study (Rehn et al., 2015), also in Sweden, followed cases up at various time periods
172 between 2.5 and 11.5 months post-*C. hominis* infection, in two locations, and found that 49% and

173 56% of cases respectively reported symptoms during this period, with fatigue, headache, abdominal
174 pain and diarrhoea being commonest. In our study, following *C. parvum* infection, cases reported

175 new-onset weight loss (30.8%), loss of appetite (25%) abdominal pain (37.8%) and diarrhoea

176 (33.3%). The figures are strikingly similar to Hunter's et al (2004) and furthermore our study suggests

177 persistence of these gastrointestinal symptoms beyond the two months studied by Hunter, and up

178 to a year after acute cryptosporidiosis. Of interest also is that certain non-gastrointestinal sequelae

179 were previously found to be significantly associated only with *C. hominis* (Hunter et al., 2004). For

180 example, new onset eye pain was reported by 5/54 (9%) of our participants in either 6MQ or 12MQ
181 in comparison with the previously reported nearly 10.9% of *C. hominis* and none of *C. parvum* cases
182 with new or worsening eye pain (Hunter et al., 2004); new or worsening joint pain was found in 22%
183 of cases in Hunter's et al. study and 33% in ours. However, interpretation of our findings requires
184 caution. Case numbers are small; there was no available control population (unlike Hunter's et al.
185 study), with cases simply acting as their own controls before and after infection; symptoms reported
186 are common among the general adult population and recall bias may also have contributed.

187

188 Medical diagnoses may underestimate the incidence of post-acute health effects. For example,
189 although 15 of 46 (33%) participants reported new onset of joint pains at 6MQ and/or 12MQ, only
190 three (20%) consulted healthcare services resulting in one new diagnosis of arthritis. Hunter also
191 found that only about a third of those reporting joint pains intended to report it to a medical
192 practitioner, and concluded that therefore the symptoms may have been relatively mild (Hunter et
193 al., 2004). Among our participants 20% disclosed a pre-existing diagnosis of IBS, considerably greater
194 than the reported UK population level of 9-12% (Jones et al., 2000). This could suggest that those
195 with pre-existing IBS may more readily present to health services, or be more likely to have
196 participated in our research study; alternatively, we cannot discount that those with IBS may be pre-
197 disposed to more symptomatic cryptosporidiosis.

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199 Four individuals without pre-existing IBS reported new symptoms that met Rome III criteria at 6MQ
200 and a further 2 at 12MQ (14% in total). This does not necessarily equate to a diagnosis of IBS
201 because other underlying pathological causes of symptoms must be excluded (by history,
202 examination or clinical investigations) before considering IBS, and two people reported alternative
203 plausible causes for their symptoms. The Rome III criteria are validated for the diagnosis of IBS, not
204 specifically for PI-IBS, and require certain symptoms to be present for 6 months or more. It could be
205 argued that duration of symptoms for diagnosing PI-IBS should be amended to 'since resolution of

206 acute infection'. If this were applied in our study, 8/43 (19%) participants without pre-existing IBS
207 reported symptoms that met these modified criteria at 6MQ – there having hardly been a chance for
208 them to have experienced symptoms of sequelae for 6 whole months. At 12MQ, 28% reported
209 symptoms consistent with Rome III criteria for IBS if those with symptom duration shorter than 6
210 months were included - twice as many as the number reporting the same symptoms for 6 months
211 or more. Two patients were diagnosed as having IBS by a medical practitioner during the 12 month
212 period following infection. In summary, we found that within twelve months following acute *C.*
213 *parvum* infection, two adults without pre-existing irritable bowel syndrome received a new medical
214 diagnosis of IBS and up to 28% (12/43) self-reported new onset of IBS-consistent symptoms. PI-IBS
215 appears to have a better prognosis than IBS diagnosed in the absence of recent known
216 gastrointestinal infection (Jones et al., 2000), and usually resolves without medical intervention.
217 Patients may gain reassurance if made aware of this potential longer term health effect and likely
218 clinical course at the time of receiving their cryptosporidiosis diagnosis.

219 The results presented here lend support to the notion of post-infectious sequelae after
220 cryptosporidiosis. In addition this is the first time non-GI symptoms have been reported following *C.*
221 *parvum*, as opposed to *C. hominis* infection. Despite the small number of participants and
222 uncertainties discussed, the outcomes from this follow-up study add to available information on the
223 self-reported and medically diagnosed health effects occurring following resolution of acute
224 cryptosporidiosis. Our ongoing work will compare self-reported symptoms among patients with *C.*
225 *hominis*, *C. parvum* and without *Cryptosporidium* infection. Quantifying post-acute health sequelae
226 assists healthcare and environmental health professionals in providing more complete advice and
227 support to patients with cryptosporidiosis in a timely manner. In turn, this may reduce subsequent
228 use of healthcare services such as repeated GP consultations and laboratory testing. Since, in the EU,
229 there is no licensed treatment for cryptosporidiosis, identifying and increasing awareness of post-
230 acute health effects may assist policy decision-makers to understand the potential longer-term
231 burden of disease and prioritise interventions to prevent *Cryptosporidium* infection.

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